Exhibit A

PROPOSAL TO BECTON-DICKINSON

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Department of Genetics

TRANSFECTION OF CHIMERIC IMMUNOGLOBULIN GENES INTO LYMPHOID CELLS

TRANSFECTION OF CHIMERIC IMMUNOGLOBULIN GENES INTO LYMPHOID CELLS

Vernon T. Oi and L.A.Herzenberg [redacted]

The objective of this project is to transfect two chimeric immunoglobulin genes into a lymphoid tissue culture cell line capable of transcribing and translating these genes into proteins. The chimeric immunoglobulin genes will be constructed using standard recombinant DNA techniques and will consist of (1) a V-D-J gene segment coding for a dansyl hapten binding V-region and a Igh-b allotype constant region; and (2) a mouse V-D-J gene segment coding for a human cell surface antigen (e.g., Leu-2) and a human immunoglobulin constant region.

THE METHOD TO DELIVER DNA INTO THE CELL. There are currently five techniques being used to transfect DNA into eukaryotic cells. All five will be examined as possible means to introduce active immunoglobulin genes into lymphoid cells. The techniques include: (1) Ca-PO4 precipitation; (2) PEG 6000 fusion of lambda phage particles; (3) vesicle fusion; (4) protoplast fusion; and (5) microinjection.

THE APPROPRIATE DELIVERY VECTOR. We have available to us suitable first generation SV40-pBR322 vectors to contain the recombinant immunoglobulin genes to be used in transfection experiments. Further development of these vectors also will be undertaken.

THE APPROPRIATE CELL HOST. Since the chimeric SV40-pBR322 vectors we are planning to use contain either the thymidine kinase or guanine phosphoribosyl transferase genes as selectable eukaryotic markers, we intend to develop lymphoid cell lines that lack these enzymes to use as transfectant recipients. These cell lines must have the potential to express immunoglobulin genes, but lack the ability to produce endogenous immunoglobulin products.

RECOMBINANT DNA. Standard recombinant DNA techniques will be used to isolate a DNS V-D-J gene segment from the genome of an existing hybridoma cell line producing anti-DNS antibodies. Igh-b constant region genes, as well as human constant region sequences will be isolated similarly. Chimeric recombinant V-D-J-Constant region sequences will be constructed from these newly isolated gene segments.

SELECTION OF TRANSFECTED CELL LINES EXPRESSING NOVEL IMMUNOGLOBULIN GENES. Should all of the above be accomplished, successfully transfected cell lines will be selected by enzyme markers (TK and GPT) and with the fluorescence-activated cell sorter using techniques and antibody reagents already developed.

APPLICAT[redacted]OR A RESEARCH OR CLINICAL INVE[redacted]TION GRANT

(Please read carefully the attached "Policies on Research and Clinical Investigation Grants" and the instructions on all pages of this form, before completing this application.)

TO: American Cancer Society, Inc.
777 Third Avenue
New York, New York 10017

Date [redacted]

Application is hereby made for a grant* in the amount of $[redacted]
for the period from [redacted] to [redacted] inclusive.

Title Expression of Transfected Mouse and Human-Mouse Hybrid Immunoglobulins

FROM:

Sherie L. Morrison, Ph.D. — Sherie L. Morrison
Name of Investigator — Signature

Associate Professor of Microbiology — (212) 694-4183
Title — Telephone No.

Microbiology — College of Physicians and Surgeons
Department — Division of Institution

701 West 168th Street, New York, New York 10032
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630 West 168th Street, New York, New York 10032
Street and Number — City, State, Zip Code

Dr. Richard J. Sohn
Name of Official Authorized to Sign for Above Institution — Signature

Director of Grants and Contracts
Title

*It is understood that each applicant, by the act of applying for a grant, agrees, if the grant is made, to abide by the Society's POLICIES ON RESEARCH AND CLINICAL INVESTIGATION GRANTS.

Columbia University
Checks to be Made Payable to
Attn. Ms. Francy, Acting Controller
Box 6, Central Mail Room
Street and Number
New York, New York 10027
City, State, Zip Code

NO. 0059.02 – 1/82

APPLICATION FOR A RESEARCH OR CLINICAL INVESTIGATION GRANT

SUMMARY OF RESEARCH PROPOSED

Name and Official Title of Principal Investigator

Dr. Sherie L. Morrison, Associate Professor of Microbiology

Name and Address of Applicant Organization

Columbia University College of Physicians and Surgeons,
701 West 168th Street, New York, New York 10032

Title of Project

Expression of Transfected Mouse and Human-Mouse Hybrid Immunoglobulins

Use this space to summarize concisely your proposed research. Outline objectives and methods. Underscore the Key words (not to exceed 10) in your abstract.

Gene transfection has become an increasingly popular method of studying gene expression. We have recently developed methods of transfecting immunoglobulin genes into myeloma cell lines; these genes are efficiently expressed. The current experiments will define the regions of the mouse heavy and light chain genes which are required for efficient transfection and those required for high level immunoglobulin expression. Once these sequences are defined we will determine the influence of their position in the molecule on their function. We also will construct novel molecules and study their expression and function. In particular we will determine if hybrid molecules with the variable region from a mouse immunoglobulin (Ig) fused to the constant region of a human Ig molecule can be effectively produced and function. Secondly, we will examine the expression and function of molecules made from gene fragment. We will see if light chain dimers, one light chain of which has a heavy chain variable region can bind antigen. Such hybrid molecules have potential therapeutic value in treating human diseases such as cancer.

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2. Aim and Method of Study:

A. Specific Aims:

The aim of these studies is to produce novel immunoglobulin (Ig) molecules by using DNA mediated transformation of myeloma cells. The project will proceed in several steps.

a. Initially we will develop an optimum transfection system and define the regions of the mouse kappa light chain gene which are important for increased transformation frequencies. We will also investigate if other Ig genes contain sequences of similar function and attempt to define the mechanism leading to the increased transformation frequency.

b. Secondly, we will define the regions of both heavy and light chain genes which are required for efficient expression. Such a definition is required to permit the rational assembly of novel molecules which will be produced at high levels.

c. Thirdly, we will produce hybrid human-mouse Ig genes, test for their efficient synthesis in transfected cell lines, and assay the biologic activity of the novel molecules. We will also attempt to produce variant proteins of altered structure and function.

B. Methods

1. Immunoglobulin Genes To Be Used

In the initial studies we will use the heavy and light chain genes from the S107 myeloma. Genomic clones of both of these expressed genes have been obtained from Dr. Matthew Scharff and are available in the laboratory. Initial expression studies (see above) have focused on the S107A kappa chain gene. We will now also construct a vector containing the S107 heavy chain gene so that we can study its expression. To date three human heavy chain genes have been acquired. A VDJ segment and the $\gamma 1$ gene have been acquired from Dr. Honjo and a kappa chain gene from P. Leder. We will initially study the expression and function of mouse V_L-human C_κ and mouse V_H-human $C_{\gamma 1}$ constructs.

2. Recipient Lymphoid Cell Lines

Our principal recipient cell line will be the mouse myeloma J558L. This produces a λ light chain, no heavy chains and transfects very well. Because λ and κ are so different structurally we anticipate little competition between these molecules in assembly with heavy chain. However, if the λ chain presents a problem we will isolate a non-producing variant of J558 using methodology which is routine in the laboratory.

3. Sequences Necessary for Efficient Transformation.

The S107A light chain gene is contained on a 7 Kb Bam Hl fragment as diagrammed in Figure 2. The deletions and partial molecules shown in Figure 2 have already been constructed and are being assayed for their transfection efficiency. Using the sites shown in the figure and others which we identify we will further assay the gene for transfection enhancement. The general protocol will be to subdivide the gene into fractions and assay each for its influence on transformation frequency. In particular we will put the Bam-Bgl or Bgl-Bgl pieces from the 7Kb L chain fragment into the Bam site of pSV2gpt and assay for transfection frequency. Other small fragments will be excised, blunt ended and Eco R1 or Bam Hl linkers put on. Bam Hl linkers have already been put on all the Hae III pieces from the L chain gene. Each fragment will be assayed for its enhancement of transformation; combinations of fragments will also be assayed to determine either synergistic or antagonistic interactions. Positive fragments will be subdivided into smaller pieces either by cutting with additional restriction enzymes or by cutting with progressive exonucleases such as Bal 31. The general objective will be to localize to as small a region as possible active sequences. The nucleic acid sequence of such regions will be determined and homologies between active regions sought.

Several possible mechanisms can be proposed to explain the increased transfection frequency: 1) replication of the plasmid as an episome; 2) increased expression of the selectible gene, in these experiments XGPT, or 3) increased integration into chromosomal DNA. We will try to distinguish among these possibilities.

Replication as an episome could be either transient during the early stages of the transfection or persistent. Transient replication increases the copy number of the plasmid within the cell and hence the probability of productive integration. To test for transient expression as an episome, 72 hours after transfection the Hirt supernatant (23) will be prepared from the transfected cell lines and the small molecular weight DNA examined by Southern (24, 25) blot after cleavage with the restriction endonuclease Mbo 1, and if available, Dpn I. Both Dpn I and Mbo 1 recognize the sequence GATC. If unmethylated this sequence is cut by Mbo I but not Dpn I; the sequence $G^{me}ATC$ is cut by Dpn I but not Mbo I. Since the dam methylase of E. coli introduces methyl groups on the N^6 position of adenine in the sequence GATC, while no eucaryotic enzymes do, it is possible to distinguish between DNA replicated in bacteria and that replicated in mammalian cells by the methylation pattern. To test for persistance as an episome, the Hirt supernatant will be isolated from stable transformants. Southern blot analysis will be done both on uncut DNA to test for the occurrence of DNA in the supercoil form and cut with restriction enzymes to assay for restriction fragments of the appropriate size. In addition, material from the Hirt supernatant will be used to transform bacteria. If replicating plasmids

are present they should be effective in transforming bacteria. If transformed bacteria are obtained, plasmid DNA will be isolated from them and the nature of the plasmid DNA determined following digestion with restriction endonucleases. In previous studies using these types of vectors, episomal replication has only been detected in Cos cells where T antigen is supplied in trans (26).

Analysis of stable transformants has already shown that the amount of gpt produced in those transfected with a pSV2gpt-S10721 is not consistently different from that produced in cells transformed using pSV2-gpt. However that does not exclude the possibility that increased transient expression of XGPT may lead to increased transformation. To test that possibility cytoplasmic extracts prepared from cells 48-72 hours after transfection will be assayed for their XGPT activity (9 and appended reprint). In the vector which we have routinely used for transfection, the SV40 early promoter has been used to drive the bacterial XGPT gene. It is also possible to use the promoter from the Herpes thymidine kinase gene (appended manuscript) to drive the XGPT gene. We will assess if sequences effective in enhancing transfection by vectors using the SV40 promoter are effective with the TK promoter and if these sequences lead to increased transient expression of sequences off the TK promoter.

It is also possible that increased transformation results from an increased frequency of vector integration into chromosomal DNA. It is difficult to directly test this hypothesis. However we will do Southern blot analysis following cleavage with restriction endonucleases with 6 base recognition sequences of DNA isolated from transformants obtained using vectors either with or lacking the enhancing sequences. This analysis will give us an estimate of the number of sites of integration per transformant. If the same size restriction fragments are found in independent transformants it will suggest a common site of integration. To confirm this it would be necessary to clone the integrated genes and directly analyze the flanking sequences. Methods to produce genomic libraries using lambda phages are available in the laboratory.

4. Identificaion of Genetic Sequences Necessary for High Level Immunoglobulin Expression.

Preliminary experiments have demonstrated that it is possible to introduce a rearranged mouse kappa light chain gene back into a mouse myeloma cell by DNA mediated transformation; the reintroduced light chain can be expressed within the myeloma cell to levels approaching that of the endogeneous myeloma light chain. Deletion analysis has also suggested that sequences within the IVS are required for efficient Ig expression. By cutting with Hind III we can now mix and match the 5' and 3' deletions. We will do these experiments to precisely define the extent of the region necessary for expression. Once we have appropriately located the sequences, we will make additional Bal 31 deletions to try and locate the sequences to within one or several

The experiments detailed about all relate to expression of the kappa chain gene. A similar series of experiments will be done to identify IVS, 5' and 3' sequences necessary for expression of the S107 H chain gene. For H chains we will also determine if the synthesis of a light chain, either specific or non-specific, is required for or facilitates expression.

To assay for the synthesis of the transfected gene product cells will be labeled with ^{14}C-valine, threonine, and leucine, cytoplasmic extracts made (27) and the Ig immunoprecipitated. Specific immunoprecipitable chains will be identified using SDS gels. We have found that the S107 kappa chain can easily be separated from the J558 lambda chain using SDS-PO_4^- gels (unpublished results). In selected experiments 2-D gels also will be used to identify the products of transfected genes (7).

The amount of the transfected product synthesized will be quantitated in two ways. Firstly, the ratio of the amount of synthesis of the endogeneous immunoglobulin light chain to the transfected light chain will be determined by scanning the autoradiograms of SDS gels of immunoprecipitates from transfected cells. If labeling is done for a short period of time so that neither chain is secreted or significantly degraded this method gives a good estimate of the relative rates of synthesis. To quantitate the synthesis as a percentage of the total protein synthesis, cells will be labeled for short periods of time (3-5 minutes) with ^{14}C-amino acids, the total amount of TCA precipitable material synthesized determined, and the amount of TCA precipitable material which is immunoprecipitated determined. Pulse chase experiments will be used to determine the rate of degradation of the immunoglobulin. Long term (3-24 hours) labeling with ^{14}C-valine, threonine, and leucine, immunoprecipitation and SDS gel analysis of the secreted product (with and without reduction) will determine what product is secreted and whether it is assembled.

Northern blot analysis and hybridization with Ig specific ^{32}P-labeled probes will be used to determine the approximate size and heterogeneity of any Ig specific transcripts in the cell lines. Formaldehyde gels and the blotting procedure of Thomas (28) is used routinely. In the cases where the recipient cell line synthesizes an immunoglobulin with the same constant region as the transfected gene, variable region probes will be used.

The 5' and 3' end of the cytoplasmic transcripts and points of splicing of the IVS will be mapped using the S1 nuclease resistance method of Berk and Sharp (29). In the case of the S107A gene the plasmid will be labeled at the Hpa I site in the constant region using T4 polymerase and the 1.5 Kb Hpa I to BAM H1 fragment used to identify the 3' end of the transcript. Label of the Hpa I site with kinase will be used to position the 3' end of the IVS and label of the Kpn site within V with T4 polymerase will be used to locate the 5' side of the IVS.

Because there is an IVS between the leader sequence and V_L and no known unique restriction site in the leader sequence, templates synthesized in M13 will be used to map the 5' end of the transcripts. Hind III linkers have already been attached to the Hae III fragment which contains the region 5' to the light chain gene and the 5' end of the variable region and should contain the light chain promoter region. This fragment will be cloned into M13, and used to synthesize message complementary probe for S1 mapping experiments. If some transcripts originate 5' of this fragment, a larger fragment will be cloned into M13. S1 analysis will be done on RNA isolated from both the transient expression experiment and from stable transformants. We have already used S1 analysis to demonstrate that the 3' ends of the mRNA from transient expression and stable transformants with many of the vectors are identical.

The Northern blot and S1 analysis will yield information about the structure of steady-state cytoplasmic mRNA. To gain some information about nuclear RNA, it will be isolated from selected transformants and the size of the nuclear transcripts determined by Northern blot analysis. Initial blotting will be done with probes which contain the entire Ig gene. Region specific probes will be used to both elucidate the pattern of processing and to identify abnormal transcripts. A necessary control for these experiments will be a careful analysis of the nuclear RNA of the recipient cell lines to eliminate the possibility that they contain aberrant transcripts of Ig genes.

5. Expression and Function of Novel Immunoglobulin Molecules

Once we have a clear idea of the sequences necessary for efficient Ig production we will begin to construct novel Ig molecules and will study their expression and function. Combinations which we will produce include:

a. [S107 kappa] + [S107 alpha]
b. [V_H S107 + γ1 human] + [S107 kappa]
c. [V_L S107 + κ human] + [S107 alpha]
d. [V_H S107 + γ1 human] + [V_L S107 + κ human]

In these constructions both the H and L chain will be covalently linked into the expression vector to increase the probability of their cotransformation and expression.

Combination a will demonstrate that it is feasible to establish an antigen binding cell line by gene transfection. Combinations b and c will demonstrate whether it is possible to get expression of hybrid molecules, and if it is possible to assemble molecules, one constant region of which is of murine origin, the other of which is human. Combination d will demonstrate if it is possible to express a molecule with the specificity of murine origin, but the constant region and effector functions of human origin.

nucleotides. The end points of the deletion will be sequenced and compared to the published sequence of the IVS (14) to accurately position them.

Once the IVS necessary for high level Ig production has been accurately identified we will do further analysis of the effects of this sequence and the structural requirements for its function. We will determine if there is a position effect on Ig production, that is, must the sequence always be at the same position and in the same orientation in the Ig gene to exert its enhancing effect. The SV40 enhancers provide an example of an enhancer that functions in various positions and orientations. The Ig sequences which failitate Ig production will be placed both 5' and 3' of their normal positions in the Ig gene and elsewhere in the expression vector in either orientation and the level of Ig expression assayed. Linkers will be put on the active fragment. By using linkers, we will invert the sequence in its normal site, and also duplicate it in both its normal and inverted orientation. Random small insertions (21) will also be put into the active sequence to define its structural requirements for function. We will make constructions with IVS consisting only of the required sequence and enough information to preserve the 5' and 3' splice junctions. In addition we will determine if the Ig sequences increase the expression of genes being synthesized off non-Ig promoters. Vectors exist with the bacterial XGPT gene being expressed using either the SV40 or the Herpes thymidine kinase (TK) promoter. The Ig sequences will be placed at various positions relative to the SV40 and TK promoters and the synthesis of XGPT assayed both in transient expression experiments and in stable transformants.

We will also test for the influence on expression of sequences 3' to the coding region. We have available a kappa cDNA clone with R1 ends. We will convert these R1 ends to BAM ends by blunt ending with S1 or T4 polymerase and adding BAM linkers. We will then exchange the 3' Hpa 1-Bam fragment from the cDNA for the same fragment from the pSV2-S107-21 vector. The resulting vector will lack sequences 3' to the mRNA. If this light chain is efficiently expressed we will do Bal 31 digestion before putting on the Bam linkers. Exchange of the Hpa-Bam fragments after Bal 31 digestion will delineate how much of the 3' sequence is required and if it is necessary to have a poly A addition site. We can add back a poly A site from SV40 to provide a new poly A site at a different position.

The sequences 5' to the gene necessary for expression will also be determined. Preliminary construction will be done by cutting with R1 + Pvu II and R1 + partial Xba, putting on R1 linkers, reclosing and assaying. Bal 31 digestion can be done before putting on the linkers to more accurately define the required sequences. The present experiments will be designed merely to identify the extent of the necessary sequences. Fine structure mapping of the promoter sequences by such methods as in vitro mutagenesis and "linker scanning" (21) are beyond the scope of the present proposal.

If we achieve efficient expression using the entire gene we will make and analyze a series using only gene fragments. Among the combinations which we plan are:

a. [V_L S107 + C_κ human] + [V_H S107 + $\gamma 1$ human with CH_1 deletion]
CH_2 deletion]
CH_3 deletion]
CH_2 + CH_3 deletion]
CH_1 + CH_2 deletion]
CH_1 + CH_3 deletion]

b. [V_L S107 + C_κ mouse] + [V_H S107 + C_κ mouse]

c. [V_L S107 + C_κ human] + [V_H S107 + C_κ human]

All transformants will be assayed, using the methods detailed above for the synthesis, assembly and secretion of Ig molecules. Transcripts will be analyzed both for their fidelity and quantity.

One of the reasons for using the S107 V_H and V_L is that they come from a molecule of known antigen specificity, an anti-phosphorylcholine antibody. Recombinant molecules will therefore be assayed for their ability to bind phosphorycholine (PC). This can efficiently be done by labeling the proteins by growing the cells in ^{14}C-VTL and then testing for binding to PC-Sepharose. The proteins binding will be analyzed on SDS gels following elution. Human $\gamma 1$ fixes complement. If recombinant molecules bind antigen, their ability to fix complement will be tested. Resistance to serum protein proteases will be tested by incubating biosynthetically labeled proteins in serum at 37°C for varying lengths of time, and then analyzing the amount of Ig which can be immunoprecipitated. Immunoprecipitated material will be run on SDS gels to determine its size. Serum half-life will be tested by injecting biosynthetically labeled proteins into mice and following their serum decay. It would be desirable to assess these parameters in humans, but such experiments are beyond the scope of this grant.

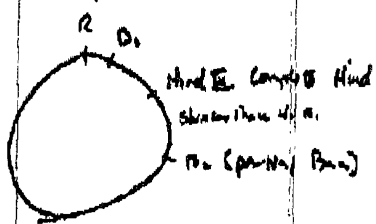
[redacted]

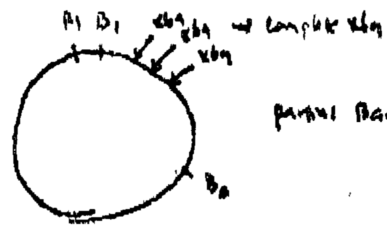
pSV2dH-SV2Hind

Hind vector Hind

Xba-Bam Vector MPC-11 Kappa

pSV2dH 9127





① Linear Hind
② Big cut B2
③ cut B2 ④ cut B1 and B2
⑤ small H-B2
⑥ small H-B1

① Xba-linear
② Big - cut B1
③ cut B2
④ cut B1 and B2
⑤ small fragments B1-Xba Xba Xba B2

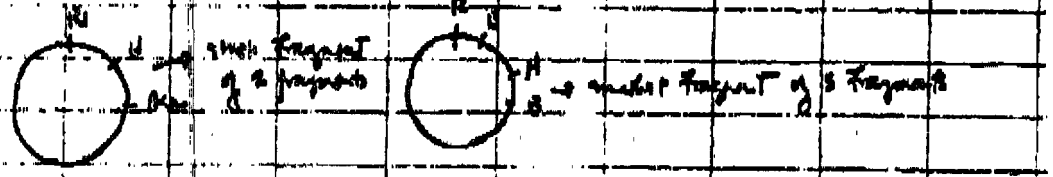
Hind in chain ng

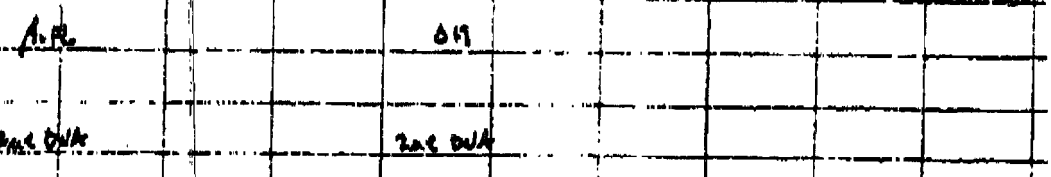
4.5 - Hind - Hind
4.65 Hind - Bam
→ should also legible as a leading + indicated 1 gene

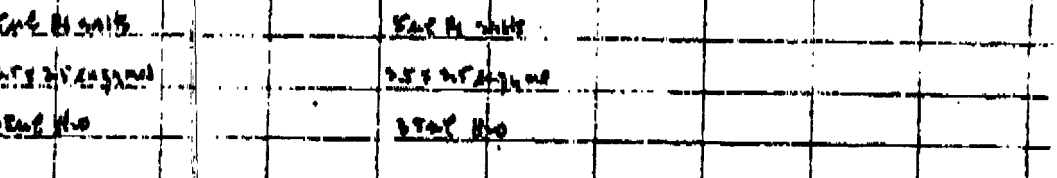
Arms: 22.7kb
9.2kb

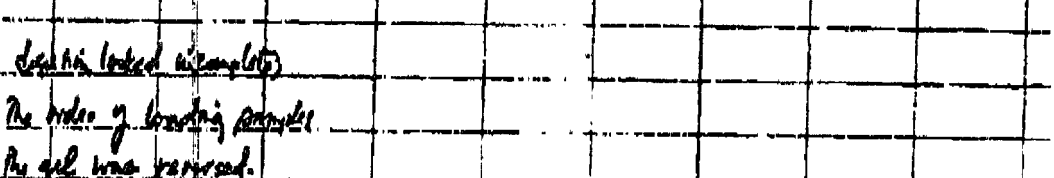
MPC-11 Kappa.
Vector 9.4kb Bam-Bam
4.4kb Bam-Xba
9.2kb Xba-Bam

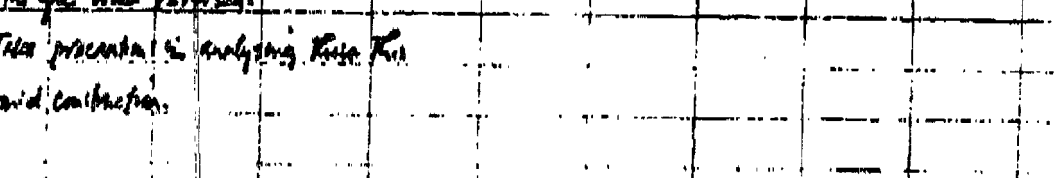
There are additional Bam Xba sites in the kappa light chain gene

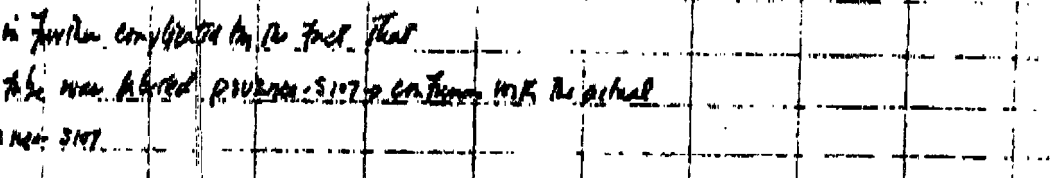


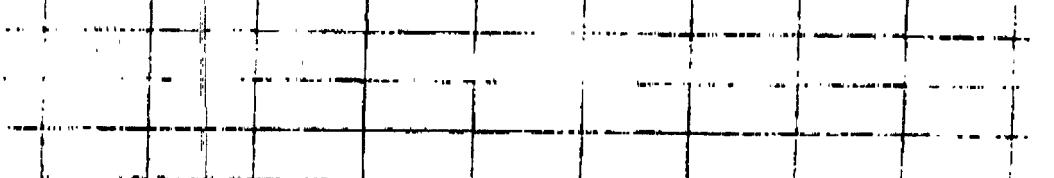












[redacted]

pSV2neo-ApPL

pSV5-neo

Neo ApPL

Neo 5107

Neo is cut with Bam-R1

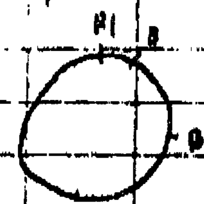
largest fragment is the vector band

Neo vector is the same as in

ApPL - cut with R1 - Bam

insert excised and should be larger than vector band - Ku worked!

5107 - cut with R1 to confirm Bam-I partial



① R1 Bam 7
② R1-Bam Bam 7.6
③ Vector
④ R1-Bam "filler"

Apparently this 5107 did not cut at all; either because it received no enzyme or because there is an inhibitor

[redacted]

Neo 9.2 µl = 9 µg

5µl R1 salts

4.5 µl enzyme

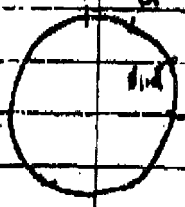
30 µl H₂O

pSV2 dH-5107K 11 µl = 4 µg

5µl R1 salts

9.5 µl R1 enzyme

30 µl H₂O



Mapping Experiment

Subject 11

[redacted] VTO started bulk O.N. E+7 + amp

[redacted] ICE 18
F5V2AH S107 VHCK A17.8
F5V2AH S107 VHCK 7
HUB 2
- process

= 18 20 mls - 8 ml (KC)
7 20 "
2 20 "
118 20 lots of prep
-20 O.N.

[redacted] spin [illegible] + RNase 10:30am
repair [illegible] 2 days

[redacted] - Harvest ICE 18 small band

			H_2O	ETOH
ICE 18	.8	mls	1.6	4.8 mls
HUB 2	1.0	mls	2.0	6.0
VHCK 17.8	.9	mls	1.8	5.4
VHCK 7	1.0	mls.	2.0	6 mls

- -20° O.N.

[redacted] - spin + wash 2 [illegible] ETOH + spin to lyphor
- [illegible]

		260	280	320	[illegible]	[illegible]	
ICE 18	1.5 ml [illegible]	.149	.072	.001	745 µg/ml	R2.06	1110
HUB 2	2.0 mls	.235	.119	.005	1175 "	R1.97	2350
VHCK 17.8	2.0 mls	.173	.084	.001	865 "	R2.05	1730
VHCK 7	2.0 mls	.168	.087	.001	840 "	R1.93	1680

Signed........ .... Date........

[redacted] 20ml O/N from plates [redacted]

HUS 1 bal. tamp 1B
HUS 2 bal "
VHCK7 bal " 〉 37° shakers
ICE Bam CAP

[redacted] 2mls from 20ml O/N into 250ml 2XYT

[redacted] process with half volumes [illegible]

- 5ml [illegible]
- 5ml [illegible]
- 1ml EDTA [illegible]
- 6.5mls [illegible]
- spin 16K 2.5hrs 11:30am ?
- phenol
- [illegible] chloro.

[redacted]
- [illegible] 10:15am
- EtOH 11:15am

VHK harvested
[redacted] spin, wash, spin, [illegible]

[illegible] [illegible] [illegible]
[redacted] OD [illegible] [illegible]

	260	280	320		
HUS 1	.097	.051	.002	R 1.9	485 ug/ml
2	.107	.058	"	1.8	535 ug/ml
VHCK7	.127	.072	"	1.8	635 ug/ml
ICE	.112	.056	.006	2.0	560 ug/ml

Signed.. Date..................

Subject............

[redacted] freeze 2.5mls O/N + 10mls DMSO + 5mls aliquot

- please make minis from 20mls O/N.
  HVS1 bal
  HVG2 bal
  VHCK7 bal
  ICE Bam

- spin 5K 5mins 15mls (HB.4)
- resusp. in gluc/lyso/sample
- 10mins R.T.
- add 2mls NaOH/SDS & mix by inverting
- 10mins on ice
- add 2mls pot. acetate/sample + mix
- 10mins on ice
- spin 7.5K 10mins HB.4
- 4mls phenol/chloro + mix + spin
  2mls " " " "
- 8mls EtOH + 5mins R.T.
- spin 7.5K 10mins HB.4
- wash c R.T. 70% EtOH
- spin 7.5K 10mins
- dry briefly
- resusp 400ul TE + 1.0ul RNase

Ligate 5:30pm ST

1 HVS1 } 30ul DNA
5ul Rxcattle
2 HVG2 } 2.0ul RI
2.5ul bal
12.5ul H2O

3 VHCK7 30ul DNA
5ul Rx cattle
2.0ul RI
2.5ul bal 1
12.5ul H2O

4-8 VHCK7 30ul DNA
5ul Rx cattle
2.5ul bal 1
12.5ul H2O

Signed............ Date............

Becton Dickinson Monoclonal Center, Inc.
2375 Garcia Avenue
Mountain View, California 94043
Telephone: (415) 968-7744

BECTON DICKINSON

CONFIDENTIAL

[redacted]

Dr. Vernon Oi
Department of Genetics
Stanford University School of Medicine
Stanford, California 94305

Dear Dr. Oi,

This letter will confirm the offer of employment extended to you for the position of Senior Scientist in the Becton Dickinson Monoclonal Center, Inc., Mountain View, California. The position primarily involves heading and establishing our new recombinant DNA laboratory at BDMC, with its major efforts directed towards the development and application of rDNA technology to immunodiagnosis and immunotherapy. You would be directly responsible to our Scientific Director, Dr. Noel L. Warner.

Your starting salary will be [redacted] which is payable semi-monthly. Also included in this offer is participation in our Bonus Plan, and Monoclonal Center Stock Option Plan. The precise details of this latter plan will need to be discussed with you on a separate occassion.

Further, you will participate in the full range of fringe benefits offered to exempt level BD employees as noted in the Compendium of Benefits statement, which I have enclosed.

For your review, I have attached a summary of the major responsibilities of the position, which you may wish to discuss with Dr. Warner.

In order to support your efforts in establishing this in-house BDMC effort in a rDNA program, we anticipate implementing this program around the start of 1984, including hiring of two Ph.D. level staff, two senior research associates, and two research associates in FY '84, with the required level of laboratory support. A new laboratory will be constructed at BDMC for this program, currently estimated to include tissue culture facilities, warm room for microbiology use and needed equipment. We estimate approximately 900 sq. feet for this purpose and approximately $200,000 for capital equipment in this current fiscal year.

Dr. Vernon Oi
[redacted]

Page 2

Becton Dickinson corporation has made a major commitment to the development in-house of a significant effort in recombinant DNA technology, with BDMC playing a key role in spearheading its significance and potential for new businesses in the biotechnology area. Hence, I am confident that the position we offer, will provide you with a significant opportunity for challenge and growth in this exciting field. If I can be of assistance or can clarify any of the foregoing, please do not hesitate to contact me.

Very truly yours,

Nagesh S.Mhatre, Ph.D.
President

NSM/mj

Attachment

P.S. This offer is valid for a period of 30 days. If you accept this offer would you please sign below and return one copy to me.

Vernon Oi

Major Scientific Responsibilities

1. To establish and supervise a research and development laboratory in the field of recombinant DNA technology, with particular relevance to the potential application of rDNA to immunoglobulins and hybridoma fields of research.

   This responsibility particularly pertains to those potential areas which are within EDMC's target goals, as related to cellular immunology, immunodiagnosis and monoclonal antibody immunotherapy.

   This will involve research efforts in:

   a) Development of chimeric immunoglobulin polypeptide chains and evaluation of the potential impact of this approach to immunotherapy;

   b) Studies on control of expression of immunoglobulin genes in target expression systems;

   c) Development of rDNA based approaches to the definition of new immunodiagnostic reagents including model studies based on three dimensional structure analysis.

2. To interact with other EDMC laboratories in assisting in their pursuit of target goals, where rDNA or other DNA related technologies (e.g. transfection) may be potentially applied.

3. Additional Responsibilities

   1. Provide appropriate input to R&D efforts in phycobiliprotein applications research.

   2. Participate in monoclonal antibody and hybridoma planning and implementation.

   3. Provide guidance and input to general corporate wide planning in potential ED utilization of rDNA technology, including establishing effective interactions with rDNA laboratory programs in other ED divisions.

BECTON DICKINSON

PERSONNEL CHANGE NOTICE

FILL IN SECTION I IN ALL CASES. Employee's Name

29	Social Security Number: 0 5 7 9 5 6 6 2 7 9	Effective Date Mo. Day Yr.: 1 2 0 1 8 3	Payroll/Clock Number:	Work Location Code Grp. Div. Sub. Dept.: 5 0 9 3 0 0 0

☒ New Hire ☐ Rehire ☐ Change ☐ Term. ☐ Leave of Absence (from & to) ☐ Retire. ☐ Death ☐ Layoff (from & to)

Line	Fields
1	Trans. Code: 1 0 1; LOA Return Date Mo. Day Yr.: ; Payroll/Clock Number: ; Last Work Date Mo. Day Yr.: ; [redacted]; (To be completed upon termination only) Y - Yes N - No
2	Job Func. No.: ; Date in Job Mo. Day Yr.: 12 01 83; Job Ch. Code: E; Job Title: SR. RESEARCH SCIENTIST; FLSA; EEO/AC No.; Shift
3	Salary Grade: 211; Scheduled Hours: 40.0
5	Type Change: N; Base Salary Old: [illegible]; New: 0 5 5,0 0 0.0 0; Period/Freq.: ANN; Amount of Change: 0 0 0 0 0 0 0 0; Date of Amount Change Mo. Day Yr.: 1 2 0 1 8 3
6	Review Date Mo. Day Yr.: 1 2 0 1 8 3
10	First Name: VERNON; M.I.: T.; Last Name: OH; Sex: M; Marital Status: S; Date of Birth Mo. Day Yr.: 0 5 1 6 4 9
12	Number and Street: 116 WAVERLEY; City: MENLO PARK; State: CA
	Zip Code: 94025; Supplemental Address: ; Home Telephone Number Area Cd. / Exch. / Number: 415 326 0341
11	Work Location Code Grp. Div. Sub. Dept.: 5 0 9 3 0 0; Date in Location Mo. Day Yr.: 12 01 83; Loc. Ch. Code: N; Spouse Date of Birth Mo. Day Yr.: ; Spouse Name:
14	BASIC GLI Code: ; Effective Date Mo. Day Yr.: 1 2 0 1 8 3; BASIC MEDICAL Code: ; Basic Effec. Date Mo. Day Yr.: 0 1 0 1 8 4
15	MAJOR MEDICAL Code: ; Major Effec. Date Mo. Day Yr.: 0 1 0 1 8 4
13	Sup. GLI Code: ; Opt.: ; Amt.:
4	Benefit Group: ; Union: ; Payroll Loc. Grp. Div. Sub.: 5 0 9 3 0 0; Code; Dental Plan Date Mo. Day Yr.: 0 1 0 1 8 4
	State Work; Last Day Worked Mo. Day Yr.; Vacation Entitlement; Workers Compensation

Employee Profile
Career Profile
Statements of Interest 101384
Published Information

REMARKS

Originator	Date	Approval	Date	Approval	Date	Personnel	Date

Sent 12/14/83

BD-2471 Rev. 6/80

Becton Dickinson Monoclonal Center, Inc.
2375 Garcia Avenue
Mountain View, California 94043
Telephone: (415) 968-7744

BECTON DICKINSON

December 19, 1983

Ms. Ginger Bryan Watters
287 G Street
Redwood City, CA 94063

Dear Ginger:

This letter will confirm our verbal offer and your acceptance of the Sr. Research Associate position, reporting to Dr. Vernon Oi, Senior Research Scientist, at the Becton Dickinson Monoclonal Center in Mountain View, California.

Your beginning salary will be $15,000 per annum, on a permanent, part time basis. You will receive a full benefits package. Working hours are from 8:45 am to 1:15 pm.

You are scheduled to have a pre-placement physical examination on Friday, January 6, 1984 at 9:30 am at the Sunnyvale Medical Clinic located on North Pastoria in Sunnyvale. Enclosed is a map to help you locate the clinic.

Welcome to Becton Dickinson and if you have any questions, or need some additional information, please contact my office at 415/ 968-7744.

Very truly yours,

Patricia Baker

Patricia A. Baker
Manager, Human Resources

PAB:jl
attachment

cc: V. Oi

MATERIAL SENT FROM L. A. HERZENBERG LABORATORY

Sent to: Sherie Morrison
Columbia, New York

Material Sent No. [illegible]
Date Sent: 11/2[illegible]
Sent By: Tim [illegible]

NOTE TO RECEIVER: Please complete the information below and return to:

Ms. Sandy Scaling
c/o Dr. L. A. Herzenberg
Department of Genetics, S-337
Stanford University School of Medicine
Stanford, CA, 94305 USA

Date Received: ____________ Material Sent No. ____________

Condition of Material When Received: ____________

Comments:

MATERIAL SENT		QUANTITY	FORM
~~Cell Line~~	purified DNA .7mls pSV2[illegible]S107 VH-Ck [illegible] 840ug/ml 6/24/85 TG		Culture ___
~~Hybridoma~~ ~~Serum~~	.25mls pSV184 DNSVH-μ 815ug/ml #3 JD		Tumor ___ Frozen ___
~~Animal (Strain)~~	Bugs pSV2AHS107HuK (from [illegible]) pSV184DNSVH-H [illegible] pSV2S107VHHuG.2 (Jacki J.)		Serum ___ Live Animal ___
Other			Other ___